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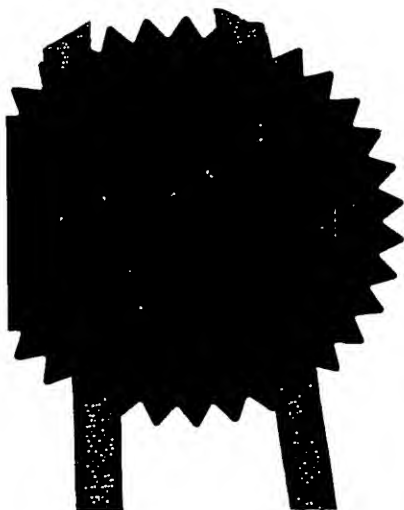
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DUPLICATE

- 1 -

DESCRIPTION

BIOLOGICAL APPARATUS

The present invention relates to an apparatus for the processing of biological material comprising a plurality of rotatable platforms that have one or more samples of biological material disposed thereon.

Advances in biochemistry and in particularly molecular biology and cytogenetics have resulted in reliable tests for certain types of diseases or conditions. Many tests require long and complex protocols and often require certain steps to be adhered to in a time dependant manner. Most tests are performed on patient samples, such as biopsies and blood samples. These samples need to be processed prior to analysis or further processing. Therefore, much of the time spent by clinical scientists is in processing samples, rather than analyzing the processed samples and producing a report of the results for the physician accurately to diagnose a disease or medical condition. Indeed, as such processing can be quite tedious and requires a high degree of skill, there is the risk that a sample may be processed incorrectly, producing a wrong result which may have serious consequences for an individual. Furthermore, a number of protocols involve hazardous chemical agents that necessitate the protocol being conducted in fume hoods/cabinets or require specialist protective clothing to be worn.

Common tests performed by clinical scientists include karyotyping of individuals for predisposition to diseases or testing whether an individual condition is related to a chromosomal abnormality such as Down's syndrome. Immunostaining can show a range of conditions in cell samples such as excess endothelial cell growth in tumour samples. Fluorescent *in situ* hybridisation (FISH) and *in situ* hybridisation

(ISH) can be used for assessing over expression of genes or proteins in cells. There are many more tests (and variations of those discussed) that are available for testing samples and these will be apparent to one skilled in the art. Most of these tests require some type of cell fixing, on order to allow ligands to be bound to the surface of a cell (or organelle) or to fix the cell onto the surface of a slide. Such fixing by its very nature often requires hazardous chemicals such as cross-linking agents or organic solvents that pose a health risk to the clinical scientist.

In order to address some of these problems outlined above and to use the clinical scientists time more effectively, a number of automated machines for processing of samples have been produced.

A flat bed apparatus is commonly found in laboratories and comprises a plurality of wells disposed on a flat horizontal bed. Reagents are then dispensed via a single tip that is linked to a motorized platform that moves in the X and Y planes servicing all the wells. The single tip often utilizes a vessel with water for cleaning the tip in between reagent additions. Such apparatus are large and take up vital bench space in the laboratory. Due to the time dependency of some steps in certain protocols, the number of wells which can be serviced can be limited by the use of one or two tips (especially if the tips has a washing step after every reagent is dispensed). There is also a tendency for the tips to become contaminated over prolonged use and tips are often prone to blocking, rendering the apparatus unusable until it is serviced. As this apparatus usually requires hazardous chemical reagents, it is often placed in a fume hood, again taking up essential experimental work space in the laboratory.

US 5,976,871 discloses a cytogenetic chamber which includes a chamber enclosure, a drying cavity in the enclosure, a door and a hand-insertion port through the

door which can maintain a substantially uniform air flow for drying of the processed material. This device is not automated and requires material to be processed by hand.

Both WO 91/13335 and US2002/0114733 discuss an immunostaining apparatus for preparing slides, having a reagent application zone and a reagent supply zone, wherein the apparatus has a carousel with two discs, one disc being the application zone the other disc being the reagent supply zone. Such an apparatus has the disadvantage that each sample is treated individually and therefore the number of samples that may be processed will be limited, should a protocol be time dependant. There is also the problem of fumes from the reagents diffusing into the surrounding area, so such an apparatus would need to be placed in a fume hood. Furthermore, the reagents need to be loaded onto the reagent supply zone manually which will be time consuming.

It is an object of the present invention to address one or more of the problems associated with the prior art apparatus and methods of processing biological samples. It is also an object of the present invention to provide an apparatus for processing biological material that can process multiple samples, which would be particularly advantageous should the samples have time dependant protocols. Furthermore, it is also an object of the present invention to provide an apparatus that can be used in confined spaces and need not be placed in a fume hood.

According to the present invention, there is provided an apparatus for processing biological materials, the apparatus comprising a plurality of stacked, rotatable platforms, each platform having a plurality of sample receiving areas located thereon, the apparatus having a first reagent dispensing member mounted for dispensing reagent to a sample receiving area on a first stacked platform and a second reagent dispensing member mounted for dispensing reagent to a sample receiving area on a second stacked platform.

each platform being rotatable to move sequential sample receiving areas thereon into orientation with the mounted reagent dispensing member to receive reagent therefrom.

The apparatus may further comprise a third reagent dispensing member mounted for dispensing reagent to a sample receiving area on a third stacked platform. Each stacked platform may have an associated reagent dispensing member mounted for dispensing reagent to a sample receiving area on each stacked platform. Preferably, the apparatus further comprises a removing member for removing waste reagent from the sample receiving area.

In accordance with the present invention there is provided an apparatus for processing biological material comprising a plurality of rotatable platforms, each platform capable of receiving one or more samples of biological material, the sample being disposed on a holding means, the apparatus further comprising a dispensing and removal means capable of dispensing one or more reagents to the sample and removing waste products from the sample.

The present invention allows multiple samples (up to approximately 40) to be processed at the same time and furthermore, due to the platform arrangement of the apparatus it occupies less space than prior art devices. Furthermore, the present invention also reduces manual operator variability and allows trained and skilled operators to be utilized more effectively in the laboratory.

Platform may mean a number of different structures such as discs or armed members and indeed any structures which would allow the apparatus to operate effectively. The sample receiving areas may be removable so that the sample is loaded outside of the apparatus.

The apparatus may be substantially contained within a releasably sealed housing. Therefore, a number of parameters can be controlled within the housing when processing is taking place. For example, the atmosphere may be controlled in order to allow for correct drying of the sample if necessary either between steps in a protocol, or at the end of processing the material. Furthermore, the housing will also allow any fumes from reagents to be contained substantially within the apparatus. The housing may also be connected to an air extraction means in order to remove fumes from the apparatus to an external source. The reagents may also be held within the housing to allow for the apparatus to be a complete processing unit. Access to the reagents may be made via a separate door disposed on the housing in order to allow for replacement and washing of the apparatus.

The platforms may rotate about a common central axis and may either rotate in unison or independently to one another. The samples will preferably be placed on standard laboratory equipment such as a petri dish or similar device. The petri dish (or similar device) will preferably be placed in the sample receiving area. The biological material may be placed on a slide or a slide cover slip and then placed onto a petri dish or similar dish. The sample receiving area may be angled relative to said platform in the range of 2° to 25° to allow liquid to collect at one part of sample. Preferably, the sample receiving area is angled in the range of 2° to 8° . Preferably, the apparatus contains from 4 to 10 platforms, e.g. from 4 to 6 platforms. Each said platform may be manufactured from aluminium, stainless steel or polypropylene, although most hard materials that are capable of resisting degradation by harsh chemical agents may also be suitable.

Furthermore, the dispensing or removing member may comprise one or more tubes. Preferably, the or each tubes are manufactured from aluminium, optionally have

a PTFE coated interior. The PTFE coating prevents any corrosive reagents from degrading the aluminum tubing. Alternatively, a PTFE tube may be disposed within an aluminum tube. Other materials or alloys may be employed in the manufacture of the tubing. The dispensing and removing member can move vertically relative to the sample receiving reagents. Preferably, movement is afforded by the dispensing and removing members rocking on a pivot in order to allow the dispensing and/or dispensing members to touch or come into close contact with the sample when the biological material is situated underneath the dispensing and/or removing members. Alternatively, movement may be afforded by the dispensing and removing members moving on a vertical rod. The vertical movement allows the dispensing and/or removing member to be lifted from the sample in order to allow the platform to rotate so that the a further sample can be processed. When the new sample is in place the dispensing and/or removing member can touch or come into close contact with the sample.

Preferably, a separate tube is provided to each reagent. It will be evident to one skilled in the art that a number of reagents may be used in the apparatus and the specific reagents will be dependant upon the protocol being implemented on the biological material. For example, in the case of processing the biological material for cytogenetic analysis, you may utilize 3 reagents: a pre-hypotonic reagent such as potassium chloride (or a standard hypotonic reagent); water to wash the sample (in between addition and removal of reagents) and a fixative such as a methanol acetic acid solution (in a 3:1 ratio of methanol to acetic acid). A given reagent may be previously mixed or may be prepared within the apparatus prior to being applied to the biological material, the latter being the case with some reagents that do not have a long shelf life. There may be multiple washing steps in order to gradually elude a reagent from a sample. For

example, multiple washing steps may be required to remove all growth media prior to applying a fixative reagent to the sample. Similarly, multiple washing steps may be required to elude neat fixative from the sample. The reagents may be dispensed and/or the waste material removed by means of peristaltic pumps. Such pumps will preferably be electronically controlled so as to ensure the correct quantities of reagent are dispensed. The removing member may comprise two or more tubes. This is advantageous, as a number of prior art devices only have one tube that may become blocked easily due to contaminants (and often fragments of glass from broken cover slips) or fragments of samples that lodge in the tubing. Providing two tubes allows one tube to become blocked without preventing the apparatus from working.

The apparatus may also have adjustable feet in order to allow the apparatus to be adjusted to suit different surface. Such feet allow small variations in levels or angles of surfaces to be overcome so that the apparatus can operate in the correct manner. Preferably a measuring device is also provided with the apparatus in order that the correct level of the apparatus can be adjusted by the user. Preferably, the measuring device comprises a spirit level or similar device. Alternatively, an electronic device which can determine the level of the apparatus is provided. The apparatus may additionally comprise a means by which the level of the feet are automatically adjusted. Preferably, there are 4 feet disposed at the 4 corners of the apparatus.

The apparatus may further comprise a sensor for detecting the presence of a sample and/or identifying the sample. Preferably, the sensor is an optical sensor. Alternatively, the sensor may be a magnetic sensor. It will be apparent to one skilled in the art that a number of sensors may be utilized in the present apparatus, such as a reflective optical sensor or a scanning laser sensor to name a few. Preferably, the sensor

may rely upon a bar code to identify the sample. The bar code may be placed on the petri dish that the sample is placed upon, or on the slide or slide cover slip. A sensor may also be provided in order to detect the characteristics of the processed biological material. It will be apparent that this type of sensor would be particularly suited to biological material that is processed in order to identify a result shown by a reporter molecule attached to a fluorescence marker, such as utilized in FISH or oligonucleotide reporter sequences. Although it is also envisaged that such a sensor may also comprise a microscope or an imaging device for post processing analysis of the sample. Supporting equipment required for the sensor, such as lighting requirements may also be provided and in order to facilitate imaging and/or the activity of the sensor, the housing can be made substantially sealed from external light.

In order to increase the automation of the apparatus, it may have an electronic control unit that controls the components of the apparatus. The components of the apparatus include the peristaltic pumps, the platforms, the dispensing and removing member, the adjustable legs, sensors and associated components. Such an electronic control unit may be programmable. Preferably, the electronic control unit is an EPROM (erasable programmable read-only memory) device. The sensors may relay information to the electronic control unit and may additionally interface with a printer and/or a computer. Therefore, the apparatus can be re-programmed with a bespoke operating protocol in accordance with different laboratories requirements. Such re-programming may be via a hand-held input device with a screen or alternatively may be via a computer linked to the apparatus.

It will be apparent to the skilled addressee that the apparatus may be used for a number of different applications for processing biological material such as FISH, ISH,

Reporter molecules, Immunostaining, Cytogenetics, High-through put screening of compounds, cell culture, cell culture optimisation experiments etc. Furthermore, the apparatus may be used for processing the material in part of a protocol or indeed used for processing the whole sample when it is in a suitable format (such as placed on a slide for example). Preferably, the apparatus is used for processing biological material for cytogenetic analysis. Processing material for cytogenetic analysis may involve processing surface culture cells in order to analyze the associated chromosomes for abnormalities or for allelic variation etc. Commonly used techniques for cytogenetic analysis is via G/Q/R/T/C-banding and NOR silver staining, although other chromosome staining and banding techniques can also be utilized. It will appear to those skilled in the art that the new techniques yet to be developed may also be used in conjunction with the present apparatus. Usually, the cells analyzed will be in cells driven to the metaphase cell cycle prior to processing, although cells in other stages e.g. interphase (FISH) may also be used.

The present invention will now be described by way of example only with reference to and as illustrated in the following figures;

Figure 1 is a perspective view of the apparatus in a closed position.

Figure 2 is a perspective view of the stacked platform within the apparatus.

Figure 3 is a perspective view of the apparatus in an open position.

Figure 4 is a exploded perspective view of the apparatus in an open position.

Figure 5 is a cross-sectional view of the dispensing and removing members.

With reference to Figures 1 to 5, there is provided an apparatus 10 for processing biological material comprising five discs 12 which are capable of holding a total number of approximately forty samples of biological material which have been placed

on a slide cover slip 13 and is placed on a 35mm petri dish 16. Each disc 12 has a number of sample receiving areas 14 which holds the petri dishes 16 in the correct position for processing by the apparatus 10. Each sample receiving area 14 is at an angle of approximately 3° relative to the disc 12 so that the petri dish 16 is inclined towards the periphery of the disc. There is also provided a lid holding area 18 on the disc 12 adjacent to the sample receiving area 14 which can hold a petri dish lid 20 whilst the biological material is being processed (as many laboratories print reference data on the lid). Each sample receiving area may be removable from the disc and replaced on locating pins 15 in order to allow the samples to be loaded away from the apparatus if needed. One part of the disc 12 has a wash station 22 for eluding excess reagents or waste. All the discs 12 are coupled to the central axis 24 which allows all the discs to rotate in unison. The central axis 24 moves in direction 26 by means of motor (not shown) which is electronically controlled. Each sample receiving area 14 on respective discs 12 are in the same position vertically. There is also provided an extraction fan (not shown) leading to an extraction tube 28 to remove fumes to the exterior of the apparatus. A number of dispensing and removal members 32 and 30 are situated in a vertical series in order for each disc sample receiving area 14 on each disc 12 can be serviced. A set of adjustable feet 34 are also provided in order to allow the apparatus to be placed on uneven or angled surfaces. The apparatus is contained within a housing 34 that has sealable doors 38 and also has a sensor 44 for detecting the presence of a sample in a sample receiving area 14.

With reference to Figure 5, the dispensing member 32 has three dispensing tubes produced from PTFE tubing disposed within aluminium tubing. Each tube dispenses a different reagent. The removal member 30 has two removal tubes produced from a

similar material to that of the dispensing tubes and allows for excess reagent and waste products after the reagent has been used to be removed from the petri dish 16. Two removal tubes are provided for the removal member in order to keep the apparatus operable should one tube become blocked. The removal tubes either rest directly onto the petri dish 16 or are very closely located near to the surface of the petri dish 16 in order to ensure maximum removal of waste material and liquid. The dispensing and removal tubes are operably joined to a pivot 40 in order that the tubes 30 and 32 can move vertically in direction 42 in order that it may allow the removal tube 30 to interface with a different sample on the same disc, when the disc 12 is rotated by means of a motor. The rocking action is linked to an electronic control unit (not shown) in order to initiate the rocking action when the disc movement is actuated.

The reagents are held in reagents vessels (not shown) into which supply tubes are inserted which are connected to peristaltic pumps that further supply the dispensing tubes 32 with reagent when required. The peristaltic pumps are also controlled electronically by the electronic control device. A waste vessel is also provided in order to receive waste material from the removal tubes, which are also connected to peristaltic pumps and controlled by the electronic control unit.

After the apparatus has been loaded with the number of samples to be processed, (the samples do not have to be loaded into sequential places if less than forty) the process can be initiated by starting the selected protocol (by selecting it on a keyboard linked to the electronic control unit). After the completed protocol has been applied to each sample the machine automatically stops, and if necessary alerts the operator. The estimated time for the completed cycle is approximately 1.5 - 2 hours for a typical protocol for cytogenetic cell processing.

Prior to the first or initial batch of samples, or at any other time between batches the complete system may be purged or rinsed out to the wash station 22 position. This facility will obviously be of use if a reagent vessel has been refilled or after an extended shutdown. The samples are loaded directly onto the disc or into the lift on/lift off sample receiving area and positioned on the discs 12 and the selected protocol initiated. The apparatus will automatically start by rotating the disc to No. 1 position and up to all 5 samples in that position are treated with the first reagent in the protocol. If the reaction time is very short the spent reagent could then be aspirated to the waste vessel by the removing tubes 30, the discs 12 then moving to position No. 2 to repeat the operation and running as necessary to position No. 8. The time required would normally be such that all additions would be completed before waste aspiration and dispersion of the second reagent. There are facilities for the sequential addition of up to six reagents although less or more than this may be used depending on the protocol that the apparatus is designed for. In some instances, two reagents may be supplied to the sample simultaneously, and subsequent removal of spent reagents as required. After the addition and removal of a particular reagent to and from all petri dishes 16, the apparatus automatically moves to the next step in the procedure, and in doing so runs through all stages required to complete the protocol. At completion the user is warned by a suitable signal (such as an audible tone or a light), and then the user manually unloads the dishes, containing the processed samples, prior, if necessary to reloading with the next batch of samples.

The apparatus is controlled by a built in program held in the electronic control device, which is re-programmable if necessary and such an electronic control device may have a number of protocols from which to chose from. The user may enter their

own protocol as wished prior to a batch being processed, via the keyboard (not shown) which is linked to the electronic control unit. Alternatively, the electronic control unit may be linked to a computer via an interface so as to re-program the apparatus.

The apparatus can be used in a number of applications and one such application is in cytogenetic analysis. In this application, the apparatus is used in order to prepare surface cell culture driven to cell cycle metaphase in the cell cycle. Cells commonly used for cytogenetic analysis include fibroblast, placental mesodermal and amniocyte cells. The protocol was used to fix the cells prior to applying a process to highlight structural features within chromosomes, such as subjecting a protocol to G-Band the chromosomes). Forty samples can be loading onto the apparatus and they are then processed through the stages from adding a suitable hypotonic solution to the final fixing with acetic acid/methanol. After, the addition and reaction of each reagent there is the facility to remove, if necessary, the spent reagent before proceeding to the next stage of the process. Both the quantity of reagent and the reaction time will be controllable by the individual operator, although all samples in one loading of the machine will be subject to the same protocol and the facility for varying the protocol between loadings is provided by means of an input device or interface with a computer which can program the electronic control unit.

The apparatus is substantially leak proof, and in addition the main logic and control circuits together with most of the actuators are mounted in a compartment separated from the liquids providing for a reliable apparatus. In the unlikely event of a leak developing, it is not going to cause a major electrical or electronic fault. The electrical system inside the main module is entirely 12 and 24 volt, fed from a power

supply mounted internally, and any fume extraction equipment is similarly organised and mounted separately from any equipment containing liquids.

The configuration of the apparatus ensures that all liquids are dispensed or removed by peristaltic pumps, which, provided scheduled maintenance is adhered to, are non-leaking. The pump delivery dose is controlled coarsely by tube size and pump rotational speed, the fine control being by programmed in relation to pump run time. A sensor 44, optical or magnetic or other detects the presence of a dish in any particular station, and only in the presence of a dish 12 does a pump dispense or remove liquid. When each operation, whether delivery or removal of liquid, at a particular dish, has been completed the dispensing tube 32, are lifted under control of the program, the disc 12 rotates to the next position that is controlled by a servomotor programmed in the required protocol, the tubes 30,32 are then lowered and the next step in the program initiated.

All liquids are stored and transferred in closed systems to reduce contamination (e.g. moisture pick up) as far as possible. The spent reagents are removed through a closed system to a waste vessel, sealed to the requirements of the user. The only position in which slides or similar cultures are exposed with reagents is in the dishes themselves and provision can be made for enclosing this volume if required. The enclosed space can be exhausted through a fume extraction system or could be subject to controlled atmospheric conditions.

Although based round a standard frame and operating procedure the exact configuration of the apparatus will be tailored to the users preferred protocol.

The machinery prefers a substantially level surface to stand on, a footprint of approximately 30x50 cms and a clear height of 60 cms, with a suitable 240 or 115 volts mains power point.

CLAIMS

1. An apparatus for processing biological materials, the apparatus comprising a plurality of stacked, rotatable platforms, each platform having a plurality of sample receiving areas located thereon, the apparatus having a first reagent dispensing member mounted for dispensing reagent to a sample receiving area on a first stacked platform and a second reagent dispensing member mounted for dispensing reagent to a sample receiving area on a second stacked platform, each platform being rotatable to move sequential sample receiving areas thereon into orientation with the mounted reagent dispensing member to receive reagent therefrom.
2. An apparatus as claimed in claim 1, wherein the apparatus further comprises a third reagent dispensing member mounted for dispensing reagent to a sample receiving area on a third stacked platform.
3. An apparatus as claimed in either claim 1 or 2, wherein each stacked platform has an associated reagent dispensing member mounted for dispensing reagent to a sample receiving area on each stacked platform.
4. An apparatus as claimed in any one of claims 1 to 3, wherein the apparatus further comprises a removing member for removing waste reagent from the sample receiving area.
5. An apparatus as claimed in any one of claims 1 to 4, wherein the apparatus is substantially contained within a releasably sealed housing.
6. An apparatus as claimed in any one of claims 1 to 5, wherein the housing is connected to an air extraction means.

7. An apparatus as claimed in any one of claims 1 to 6, wherein the platforms are rotatable about a common central axis.
8. An apparatus as claimed in any one of claims 1 to 7, wherein the platforms rotate in unison.
9. An apparatus as claimed in any one of claims 1 to 7, wherein the platforms rotate independently.
10. An apparatus as claimed in any one of claims 1 to 9, wherein the sample receiving area is angled relative to said platform in the range of 2° to 25° to allow liquid to collect at one part of sample.
11. An apparatus as claimed in any one of claims 1 to 10, wherein the apparatus contains from 4 to 10 platforms.
12. An apparatus as claimed in any one of claims 1 to 11, wherein the dispensing or removing member comprise one or more tubes
13. An apparatus as claimed in claim 12, wherein the or each tube is manufactured from aluminium, optionally having a PTFE coated interior.
14. An apparatus as claimed in claim 12, wherein the or each tube comprises a PTFE tube disposed within an aluminium tube.
15. An apparatus as claimed in any one of claims 1 to 14, wherein the dispensing and removing member can move vertically relative to the sample receiving area.
16. An apparatus as claimed in any one of claims 11 to 15, wherein separate tubes are provided to dispense different reagents.

17. An apparatus as claimed in any one of claims 1 to 16, wherein the reagents are dispensed and/or the waste material removed by means of peristaltic pumps.
18. An apparatus as claimed in any one of claims 1 to 17, wherein the apparatus has adjustable feet in order to allow the apparatus to be placed on surfaces that in have an incline.
19. An apparatus as claimed in any one of claims 1 to 18, wherein the apparatus further comprises a sensor for detecting the presence of a sample and/or identifying the sample.
20. An apparatus as claimed in claim 19, wherein the sensor is an optical sensor.
21. An apparatus as claimed in claim 19, wherein the sensor is a magnetic sensor.
22. An apparatus as claimed in either claim 19 or 20, wherein the sensor relies on a bar code to identify the sample.
23. An apparatus as claimed in any one of claims 1 to 22, wherein the apparatus further comprises a sensor for detecting characteristics of the processed biological material.
24. An apparatus as claimed in any one of claims 1 to 23, wherein an electronic control unit controls the components of the apparatus.
25. An apparatus as claimed in claim 24, wherein the electronic control unit is programmable.
26. An apparatus as claimed in claim 23 or 25, wherein the sensors relay information to the electronic control unit.

27. An apparatus as claimed in claim 26, wherein the electronic control unit can interface with a printer and/or a computer.
28. An apparatus as claimed in any one of claims 1 to 27, wherein the apparatus can process approximately 40 samples.
29. An apparatus as claimed in any one of claims 1 to 28, wherein the apparatus is used for processing biological material for cytogenetic analysis.
30. An apparatus as claimed in any one of claims 1 to 29, wherein the apparatus is used for processing surface culture cells in order to analyse the associated chromosomes.
31. An apparatus as claimed in any one of claims 1 to 30, wherein the apparatus is used for processing surface culture cells in order to analyse the associated chromosomes.

ABSTRACT

The present invention relates to an apparatus for processing biological materials, the apparatus comprising a plurality of stacked, rotatable platforms, each platform having a plurality of sample receiving areas located thereon, the apparatus having a first reagent dispensing member mounted for dispensing reagent to a sample receiving area on a first stacked platform and a second reagent dispensing member mounted for dispensing reagent to a sample receiving area on a second stacked platform, each platform being rotated to move sequential sample receiving areas thereon into orientation with the mounted reagent dispensing member to receive reagent therefrom. The present invention is particularly suited to processing biological material for cytogenetic analysis.

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Figure 1

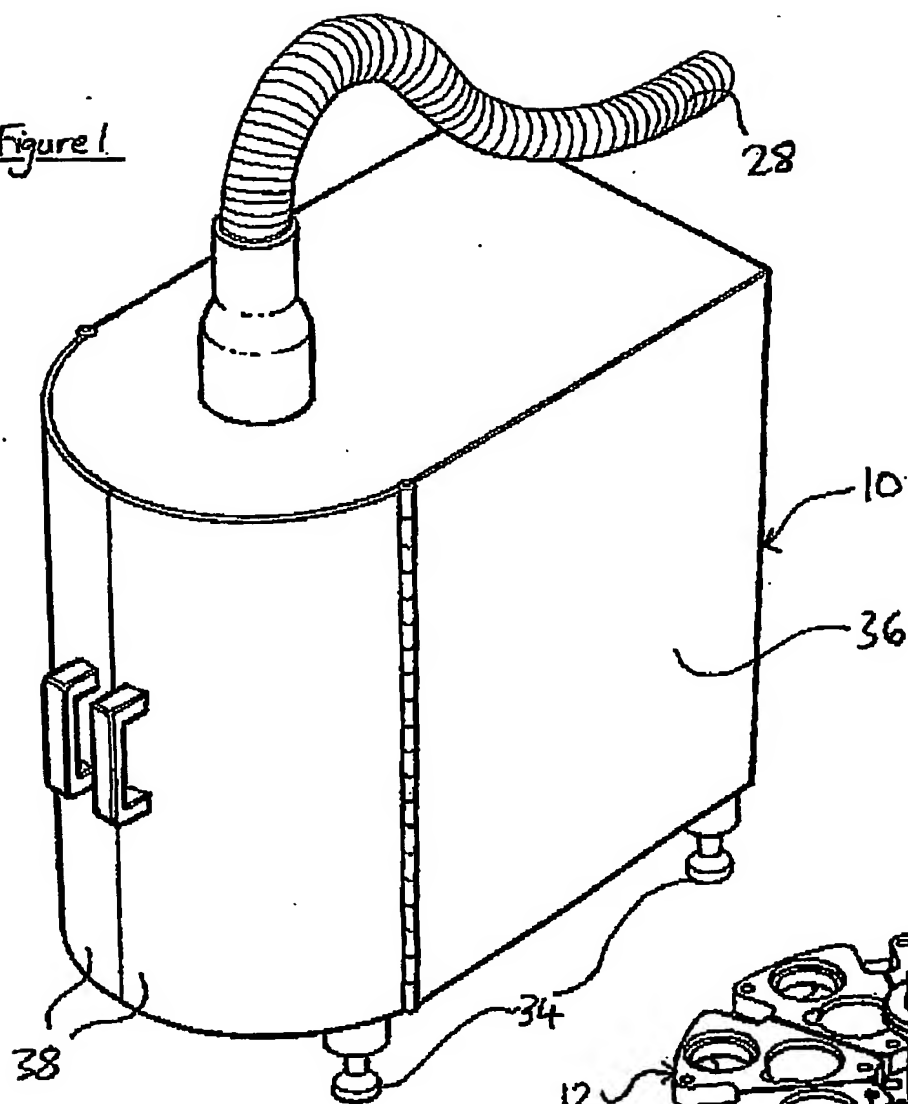
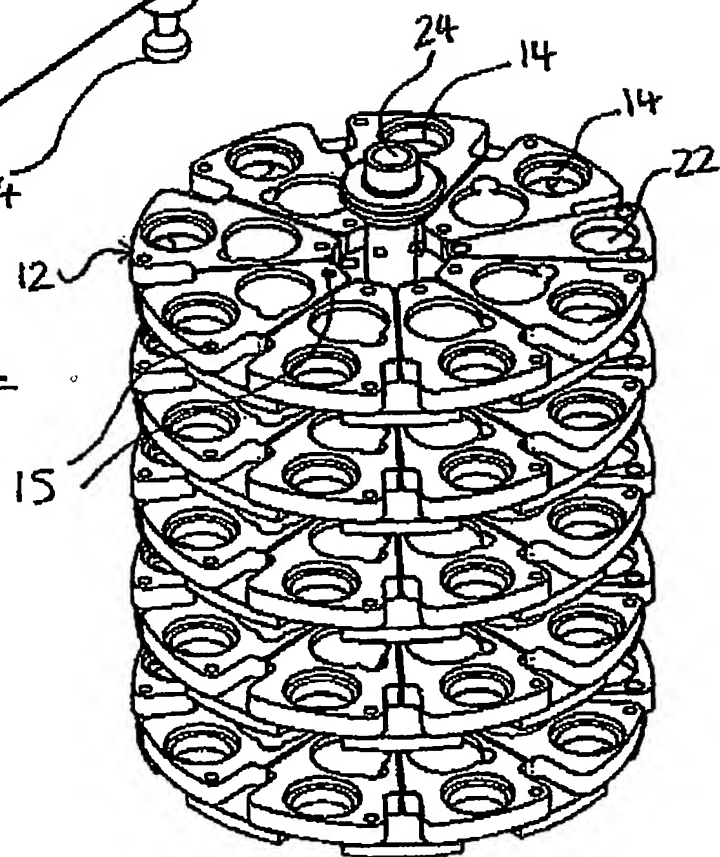
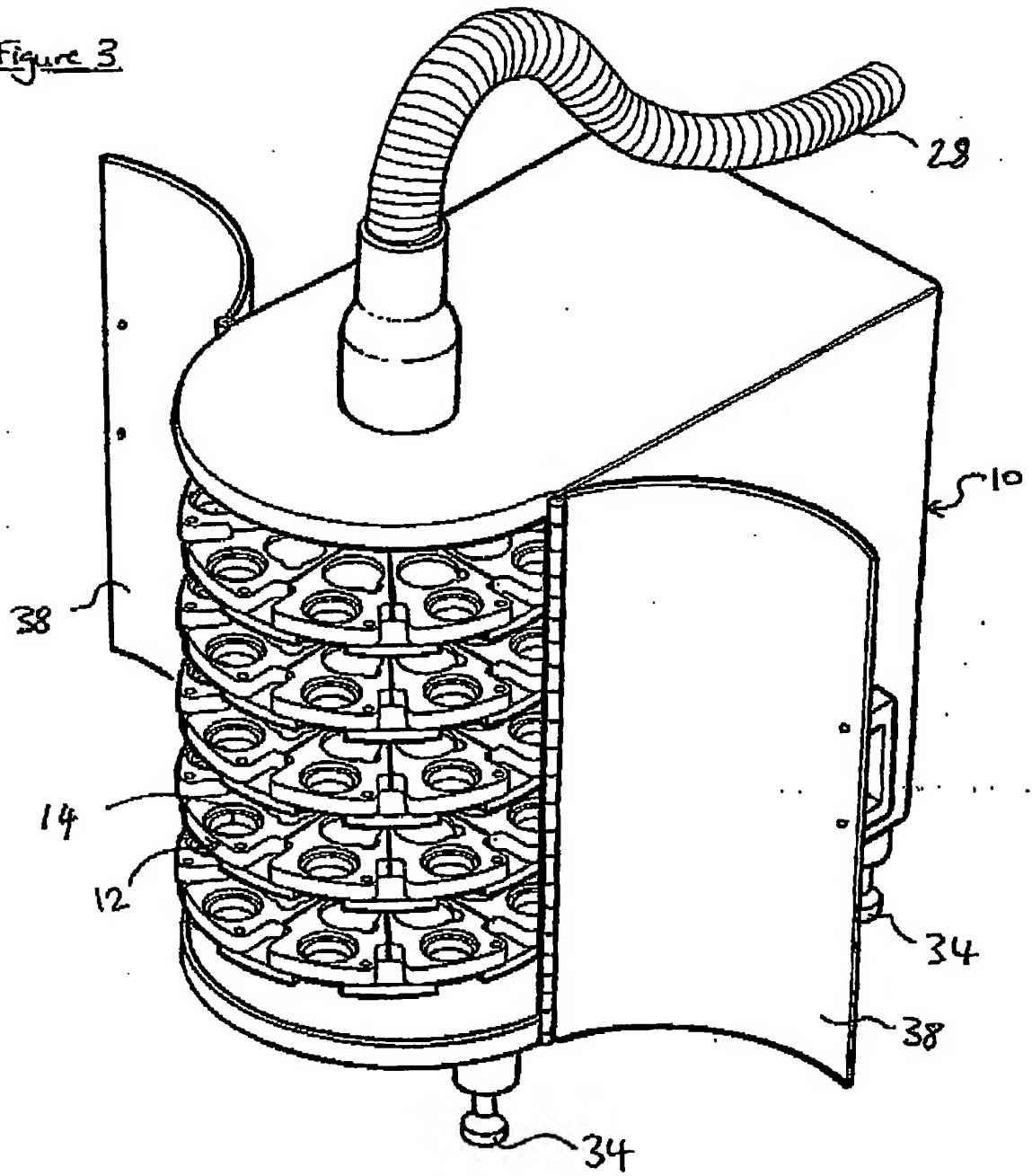


Figure 2



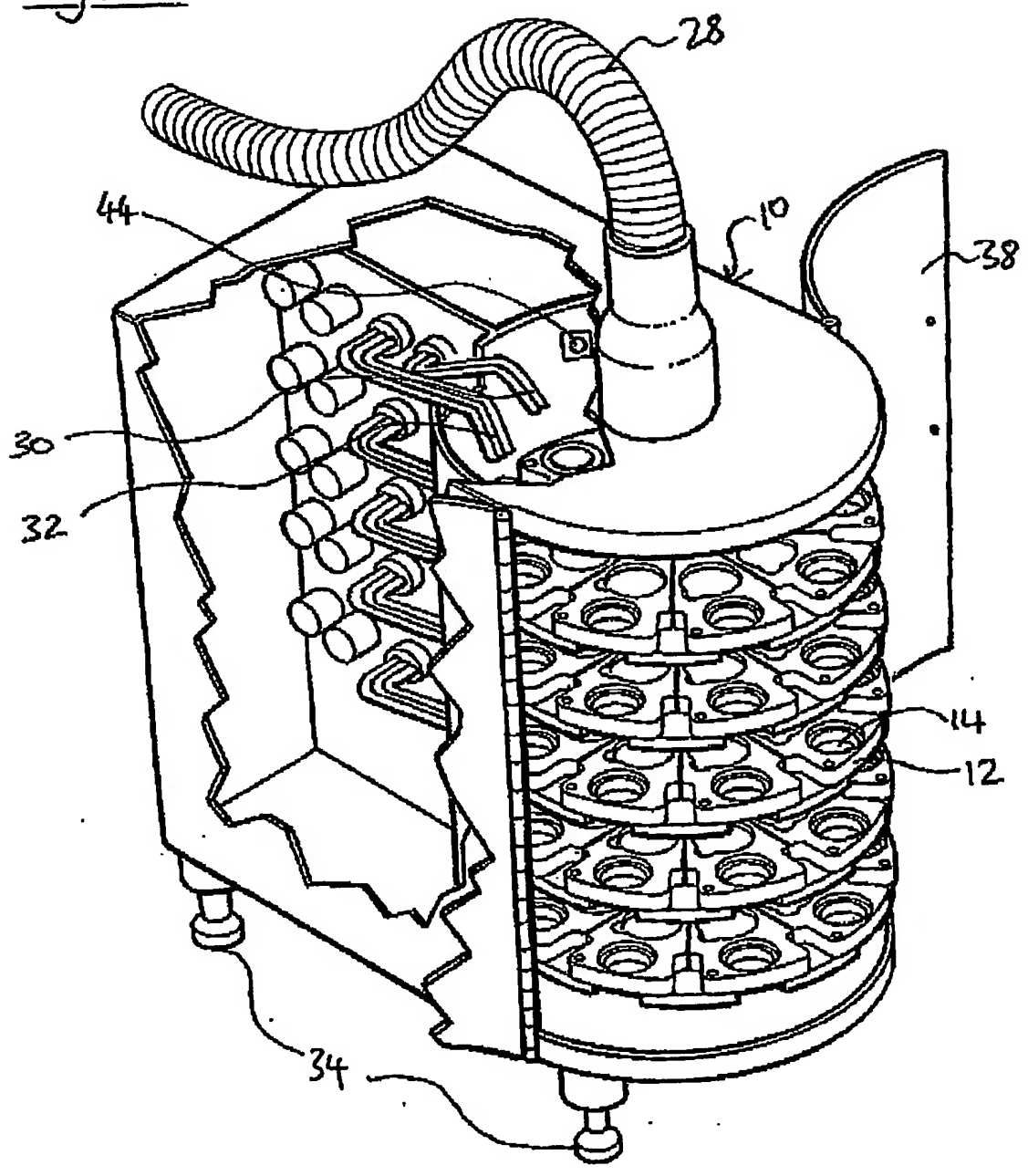
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Figure 3



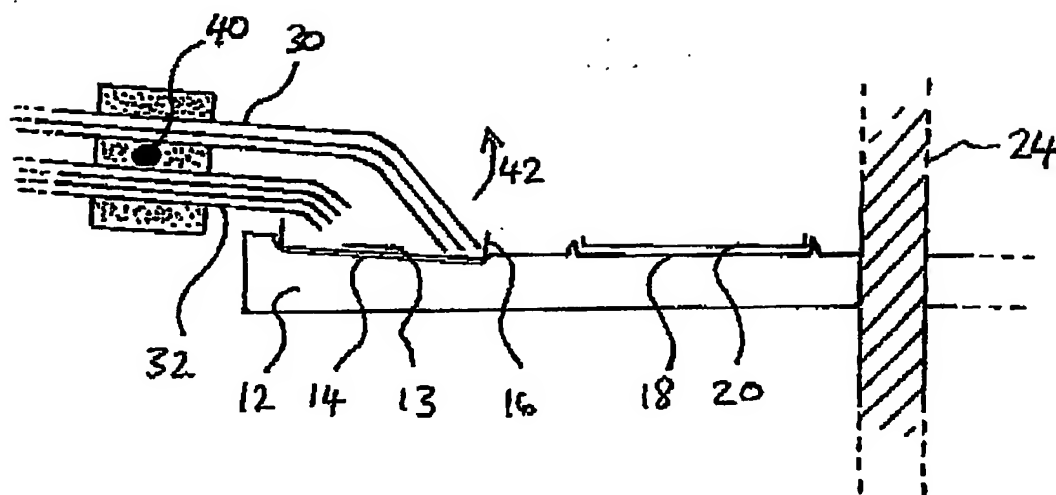
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Figure 4



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Figure 5



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